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The Effects of Changing Intake and Supply Air Flow on Biological Safety Cabinet Performance

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The operational air flow balance recommended by the Class II biological safety cabinet (BSC) manufacturer is called the nominal setpoint. Few, if any, BSCs actually operate at nominal setpoint under normal laboratory conditions. The purpose of this article is to define statistically the air flow boundaries within which a BSC will perform adequately. Performance is quantified by the microbiological aerosol tracer test as defined in the National Sanitation Foundation Standard #49. The boundaries which delineate passing test results from failing are called the performance envelope. Performance envelopes for three different models of BSCs are reported. They were determined by combining test data from at least five individual cabinets from each model. An ad hoc statistical method for calculating confidence limits around the performance envelope lines was developed to enhance the interpretation of conflicting performance envelope data. Results indicated that each model of BSC has its own unique performance envelope in terms of size and shape.

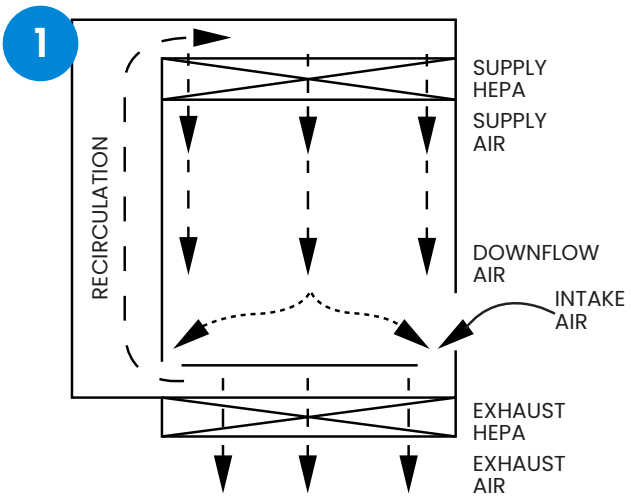
Additionally, the magnitude of the standard deviation of the air flow balance data from the performance envelope boundaries varies from one model to another. This indicates that there is a potential for any single microbiological test to be a misrepresentation of a generalized pass—fail situation, and that potential varies from model to model. Replicate microbiological aerosol tracer testing must be done over a wide range of air flow balance setpoints to ensure BSC performance under laboratory conditions. Jones, Jr., R.L.; Stuart, D.G.; Eagleson, D.; Greenier, T.J.; Eagleson, Jr., J.M.: The Effects of Changing Intake and Supply Air Flow on Biological Safety Cabinet Performance. Appl. Occup. Environ. Hyg. 5:370-377; 1990.

INTRODUCTION

Users of Class II biological safety cabinets (BSCs) expect this equipment to provide protection against the agents with which they work (personnel protection) and to help protect their work from contamination (product protection). [1-6] There are several types of Class II BSCs designed to handle differing laboratory activities and situations. [6-8]

Class II BSC performance depends on control of certain design elements that are common to all the various cabinet types. These elements include a blower in addition to the generic characteristics shown in Figure 1. [8] Cabinet design and manufacturing and testing requirements help minimize the potential for a significant amount of hazardous agent escape through the physical structure of the unit. There are at least two high-efficiency, particulate-arresting (HEPA) filters per unit. A supply HEPA filter cleans the air entering the work area, and the exhaust HEPA filter cleans the exhaust air. The air flow pattern consists of clean downflow air that sweeps aerosols along as it descends through the work area. The aerosol-bearing downflow air splits as it nears the work surface. The back portion exits the work area via the rear suction grill. That air moving downward in the front of the work area joins the intake air. These two air streams work in concert to form an air barrier at the work access opening as they flow down into the front suction grill. Depending on cabinet design, a percentage of the total air flow volume may be recirculated through the supply HEPA filter.

Figure 1



Generic characteristics of a Class 11 biological safety cabinet. Symbols: X, HEPA filter; ---- , supply air;– – – –, cabinet; ——, downflow air; ·····, cabinet intake air; — - —, cabinet exhaust air.

Continued BSC performance requires periodic certification. The cabinet carcass and the HEPA filter installations must not leak. The intake and supply air flow volumes must remain in proper balance for the work access opening air barrier to minimize egress or ingress of particulates. [8]

Standards, [3,6,9] guidelines, [2, 10-12] and purchase specifications [1,4,5] recognize the importance of proper air flow balance in this equipment. The specific downflow and intake velocities assigned to each model by the manufacturer for normal BSC operation are known as the nominal setpoint. These nominal setpoints are usually established by finding a single air flow balance point at which a representative unit of a given cabinet design passes the microbiological aerosol tracer tests. [1,4-6] These performance tests are run under ideal conditions on cabinets which have been certified, with carefully calibrated equipment, to be operating according to the manufacturer's specifications.

There are many less than ideal conditions in the working laboratory that force operational air flow balance in BSCs to wander from the established nominal setpoint. For example, variability in instrument calibration results in cabinets being set at air flow setpoints outside of intended parameters. Variations in volumes of air supplied to or extracted from the room can significantly change the operational air flow balance point of a BSC where cabinet exhaust air is ducted to the outdoors. Line voltage variations and uneven filter loading affect the balance of intake to supply air volumes as well as the total flow of air through the cabinet. [8] Cross drafts also influence cabinet performance. [13]

Confidence that a cabinet will perform acceptably under such conditions depends on information concerning how far out of balance that cabinet's air flow can wander before losing performance as determined by the microbiological aerosol tracer tests. Consequences of intake and supply air flow drifting away from the nominal setpoint are depicted in Figure 2. Too little air flow results in the cabinet passing neither the personnel nor the product protection tests (Figure 2B). Low intake air flow with high supply air flow compromises personnel protection (Figure 2D).

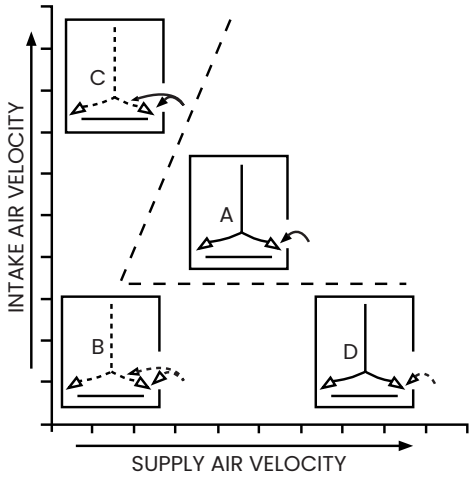
High intake air with low supply reduces product protection (Figure 2C). The purpose of this article is to reveal ranges of air flow setpoints within which different cabinet models pass the microbiological tracer tests. The range of extreme air flow setpoints within which a particular BSC model passes the microbiological aerosol tracer tests defines that cabinet's performance envelope.

METHODS AND MATERIALS

The exhaust air from all cabinets was vented to the outdoors via a dedicated in-house exhaust system equipped with a thin-plate orifice and inclined manometer system to measure exhaust air flow volume. The air flow in the cabinets was forced out of balance (away from the nominal setpoint) by adjusting line voltage to the cabinet and by varying the cabinet exhaust air volume exiting via the building exhaust system. Calculated average intake air velocity in meters per second (m/s) or feet per minute (fpm) was derived from the pressure drop, in inches of water column, across a calibrated 13.3-cm (5.25-in.) thin-plate orifice read on a 12.7-cm (5-in.) inclined manometer (Dwyer model 424). This pressure drop was converted to cubic meters per second (m/s) or cubic feet per minute (cfm) using conversion tables. [14] This volume was divided by the area of the work access opening to yield the calculated average intake air velocity. In cabinets with full supply air diffusers, downflow air velocities were measured on a 15-cm x 15-cm (6-in. x 6-in.) grid pattern at the bottom of the window frame. The individual readings were averaged to yield the average downflow (supply) velocity. In cabinets with partial supply air diffusers, providing faster air behind the view screen, supply air velocities were measured on a 10.2-cm X 10.2-cm (4-in. x 4-in.) grid 10.2 cm (4-in.) below the supply filter with the diffuser removed in order to reflect the actual supply air flow volume delivered. These readings were averaged to yield the average supply air velocity. A ring stand and a grid pattern were used with the thermoanemometer (Alnor model 8500 calibrated traceable to the National Bureau of Standards) to facilitate reproducibility of the readings.

Performance of the cabinets was measured under various imbalanced air flow conditions using the following modifications of the microbiological aerosol tracer tests for personnel and product

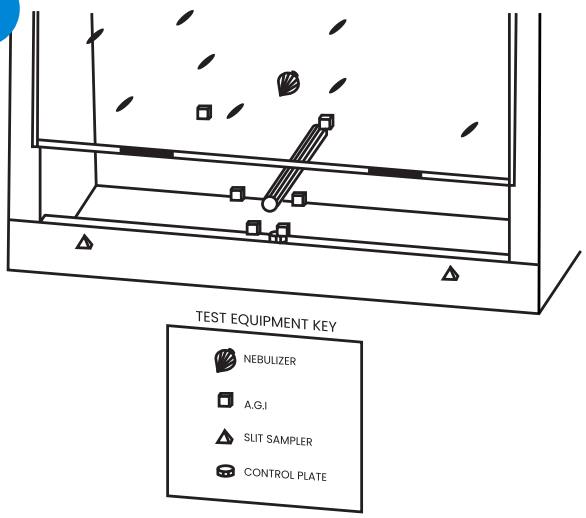
Figure 2



Performance envelope concept. Symbols: — —, performance envelope; - - - - - , low air flow; ==, high air flow; ——, nominal air (A) nominal setpoint passes product and containment tests. (B) Low intake and downflow volumes result in poor product and containment protection. (C) Low intake and high downflow volumes yield poor containment. (D) High intake versus low downflow volume results in poor product protection.

protection described in National Sanitation Standard #49. [8] The Collison nebulizer (CN-8, BGI Inc., Waltham, Massachusetts) was calibrated to deliver no less than 2.5 x 108 recoverable Bacillus subtilis spores per 5-minute delivery time for the personnel protection tests. Appropriate dilutions of the spore suspension were made to yield no less than 2.5 x 106 per 5-minute run time for the product protection tests. Nebulizer runs were 15 minutes (equivalent to three standard National Sanitation Foundation 5-minute runs), Passing criteria for the personnel

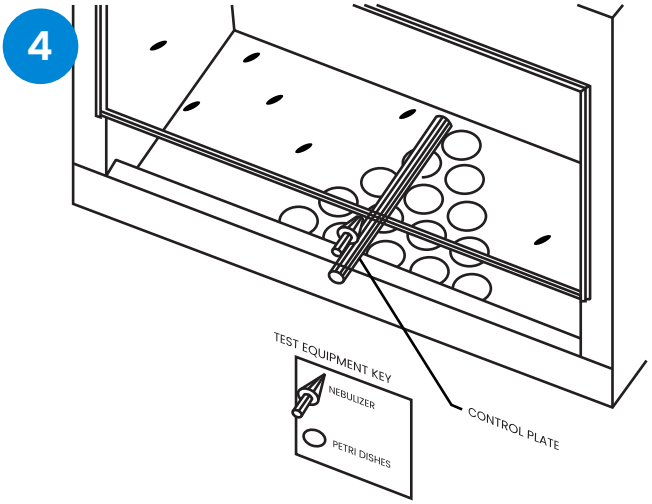
Figure 3



Schematic of the microbiological containment test.

protection runs were no more than 30 colony forming units (CFU) recovered from all 6 all glass impinger samplers (AGIs) and no more than 5 CFU on both slit sampler plates (Figure 3). The passing criterion for the product protection test was no more than 15 CFU recovered on all the work surface plates per run. These plates were arranged in a triangular pattern (5, 4, 3... starting at the front of the work surface) centered under the stainless steel cylinder for the product protection test (Figure 4).

Figure 4



Schematic of the microbiological product protection test.

At least five cabinets of the same model were microbiologically tested at various imbalanced air flow setpoints. These setpoints were plowed on x and y axes as supply or downflow air flow velocity in m/s (fpm) versus intake air flow velocity in m/s (fpm) and labeled pass or fail according to the microbiological test results. Separate performance envelope lines were drawn through the data points dividing the passes from failures for product and personnel protection tests. These straight lines were mathematically adjusted so that they were centered between the line adjusting points (the farthest fail point on the pass side and the farthest pass point on the fail side) (see Figure 6). The product protection line's fail side was to the left and the pass side was to the right of the line. For the personnel protection (containment) line, the fail side was below and the pass side was above the line. If all passes were on one side of the line and all failures on the other, too little data were available for the following ad hoc statistical procedure.

Once a product or containment line was adjusted, the equation for the line was established in order to calculate the distances between data points and the line. These distances were used to calculate the standard deviation of the data.

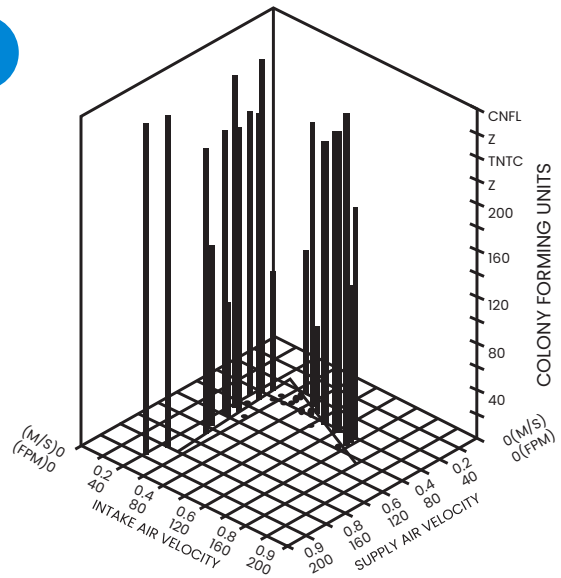
The standard deviation (σ) of data for each line was derived from those data points whose distances from the line were ≤ the line adjusting points (see Figure 6). The standard deviation was calculated by averaging the squares of the distances of the data points from the line and taking the square root of that average:

$$\sigma = \sqrt{\frac{\Sigma x^2}{n-1}}$$

where: n = the number of data points
x = the distance from the data point to the line

Parallel lines were drawn on both sides of the product and containment lines at distances equal to the second standard deviations of the data (refer to Figures 6-8). The first, second, and third standard deviations of the performance envelope data yieid confidence levels of 68, 95, and 99.7 percent, respectively.

Figure 5



Three dimensional illustration of the abrupt borders of a cabinet performance envelope. Symbols: ——-, performance envelope line; — N —, too numerous to count (TNTC); — N - N —, confluent (CNFL).

RESULTS

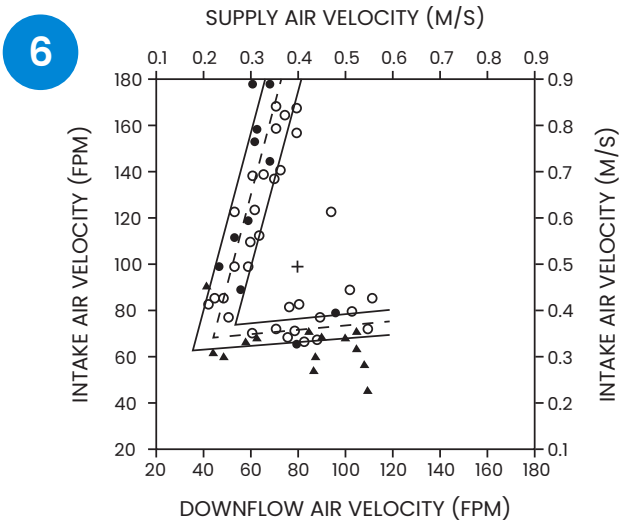
When the intake air flow was raised while keeping the supply low, a line could be drawn, to the right of which the cabinet passed both tests and to the left of which the cabinet failed product protection (Figure 2). Failure of individual cabinets to pass the bacterial aerosol tracer tests for containment and product protection were clear cut at the outer boundaries of the performance envelope. A cabinet that passed the microbiological test with ease inside the performance envelope line failed miserably [too numerous to count (TNTC) or greater] barely outside the performance envelope line (Figure 5). Nevertheless, when plotting performance envelopes of several individual cabinets from the same model, the distinct line of a cabinet performance envelope becomes a zone of intermingled pass/fail points. This zone describes the performance boundaries for that particular cabinet model (Figures 6-8). To quantify the outer boundaries of the performance envelope in terms of confidence limits, the ad hoc statistical method was applied to data of three different cabinet models (Figures 6-8).

The performance envelope shown in Figure 6 represents data collected from five different cabinets of the same model. Statistical analysis of the performance data reveals a 95 percent confidence limit span of 0.082 m/s (16.1 fpm) for the product line and 0.050 m/s (9.8 fpm) for the containment line. Line adjusting points were 0.029 m/s (5.7 fpm) and 0.021 m/s (4.2 fpm) from the product and containment performance envelope lines, respectively. The number of test points selected by the line adjusting points was 26 for the product line and 17 for containment. The nominal set point was 0.142 m/s (28 fpm) for both the product and containment lines.

The data shown in Figure 7 represent five different cabinets of a second model. The spans of the model's 95 percent confidence limits were 0.032 m/s (6.4 fpm) and 0.044 m/s (8.5 fpm) for the product and containment lines, respectively. Line adjusting points were 0.012 m/s (2.4 fpm) from the product Hine and 0.021 m/s (4.1 fpm) from the containment line. The line adjusting points isolated product and 20 containment data test points. The distances from the nominal set point to the performance envelope's product and containment boundaries were 0.335 m/s (66 fpm) and 0.264 m/s (52 fpm), respectively.

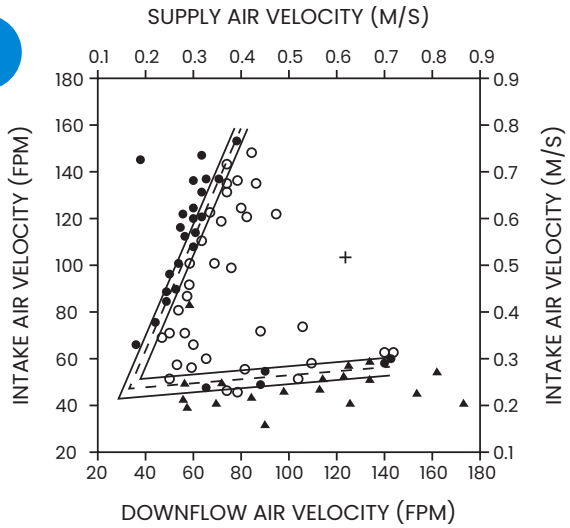
Data shown in Figure 8 were collected by testing seven different cabinets of a third model. The statistics revealed that the 95 percent confidence limit span was 0.108 m/s (21.3 fpm) for the product line and 0.104 m/s (20.5 fpm) for the containment line. Line adjusting points for product and containment lines were 0.043 m/s (8.4 fpm) and 0.046 m/s (9.1 fpm) from the respective lines. They isolated 35 product and 24 containment data test points. The nominal set point was 0.112 m/s (22 fpm) from the product line and 0.279 m/s (55 fpm) from the containment line.

Figure 6



The performance envelope derived from the biological test data for model I. Symbols: ○, passed product & containment test: ●, failed product test; failed containment test; line adjusting points; +, nominal setpoint; - - - - , performance envelope, ——, 95% confidence limit lines.

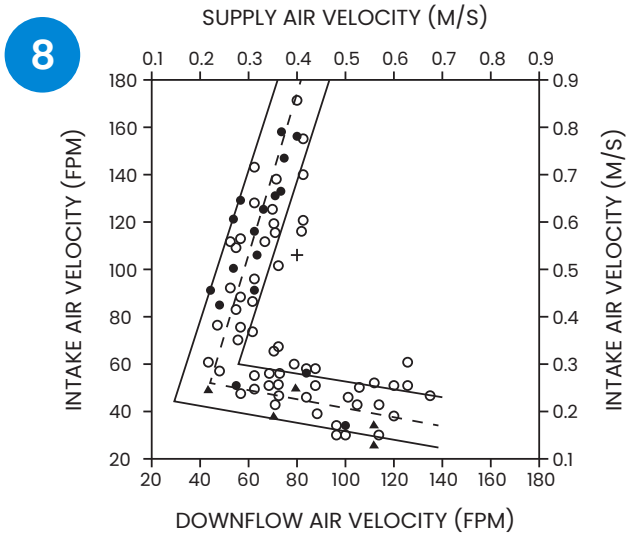
Figure 7



The performance envelope derived from the biological test for model II. Symbols: ○, passed product & containment test: ●, failed product test; failed containment test; line adjusting points; +, nominal setpoint; - - - - , performance envelope, ——, 95% confidence limit lines.

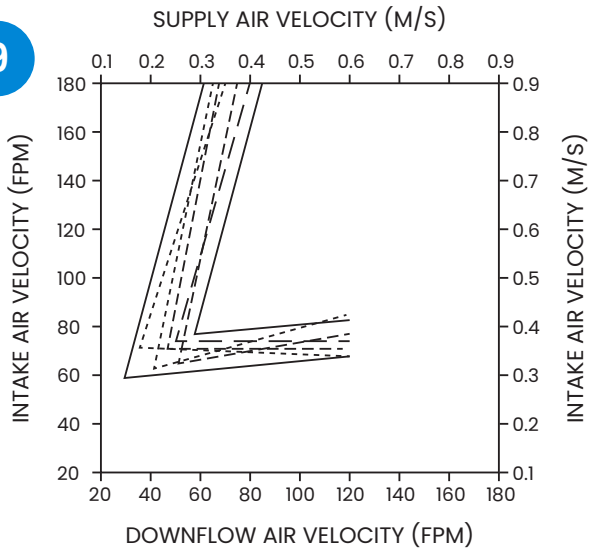
Comparing the five individual cabinet performance envelopes of the model in Figure 4 to its 95 percent confidence limit lines resulted in only a small portion of an individual performance envelope Hine falling outside of the model's 95 percent confidence limits (Figure 9).

Figure 8



The performance envelope derived from the biological test data for model III. Symbols: ○, passed product & containment test: ●, failed product test; failed containment test; line adjusting points; +, nominal setpoint; - - - - , performance envelope; ——, 95% confidence limit lines.

Figure 9



Individual cabinet performance envelopes of model I versus the 99.7% confidence limits of the model. Symbols: - - - - -, each individual performance envelope; ——, 99.7% confidence limits of models I's performance envelope.

DISCUSSION

The microbiological aerosol tracer test is the one actual performance criterion against which Class If BSCs are measured. The periodic certifications of proper cabinet operation (filter leak, pressure test, and air flow balancing) are only indirect indications of whether or not the cabinet will actually provide adequate personnel and product protection. After certification, however, users of BSCs generally assume that these cabinets will perform to the criteria of the microbiological tests while they are in use.

The only actual performance check on a cabinet model required by the industry standard is the microbiological aerosol tracer test run at nominal setpoint by the National Sanitation Foundation once every five years. This microbiological testing of a cabinet at the nominal setpoint only indicates whether the nominal setpoint is within the performance envelope, but the test provides no information as to its location within the envelope.

Determination of the performance boundaries of a given Class II BSC design can be accomplished by microbiological aerosol testing of several cabinets of the same model at various extreme air flow settings. The test results identify air flow balance setpoints at which the cabinet passes or fails the personnel and product protection tests (Figures 6-8). Straight lines can be drawn to separate the majority of the passes from fails. The 95 percent confidence limit lines can be drawn by calculating the second standard deviation of the distances from each of the lines to the data points isolated by the line adjusting points.

The breadth of the confidence limit lines for each performance envelope line is attributable to the span between the line adjusting points. The percent confidence limit of a performance envelope indicates the probability that a fail point will not migrate beyond the inner confidence limit line and a pass point will not migrate beyond its outer

confidence limit line. If a user requires 99.7 percent confidence that a cabinet performs up to the industry standards, then that model of cabinet must operate at an operational air flow balance setpoint inside the inner limits of the 99.7 percent confidence range of its performance envelope. If the cabinet's air flow balance setpoint was between the 99.7 percent confidence limit lines, the probability of the cabinet performing up to the industry standards would be between 0.3 percent and 99.7 percent depending on the setpoint's exact location between the confidence limits lines.

It is important to note that each model of Class II BSC has its own unique performance envelope with respect to size, shape, and location of the nominal setpoint within it (Figures 6-8). These performance envelope characteristics are important in determining an optimal nominal setpoint for each model of cabinet. A large performance envelope is insignificant if the nominal setpoint is located near one of the envelope boundaries. In this situation, a minute change in laboratory conditions could compromise cabinet performance.

Individual units of a given model have envelopes reproducible within the statistical confidence limits of particular model (Figure 9). This appears to support an industry wide assumption that biological test results can be reproduced in cabinets from the same model. However, the degree of reproducibility of individual cabinet performance envelopes varies from model to model (Figures 8). This is borne out by the spans of the various 95 percent confidence limits in each of the models tested.

The microbiological testing of a cabinet at just one set of air flows only indicates whether the setpoint is within the outermost boundary of that model's 99.7 percent confidence limit lines. Therefore, repeatability of biological test results for any one cabinet depends on the location of the air flow balance setpoint in relation to that model's performance envelope confidence limit lines. Together with the realization that all standard microbiological testing is performed under ideal laboratory conditions, this magnifies the need for optimizing the nominal setpoint within the performance envelope. Unless the envelope is defined before the setpoint is determined, there is little confidence that the cabinet will perform properly while in use.

Normal laboratory conditions such as fluctuating voltage [8] erratic in-house exhaust systems, and cross drafts [13] will compromise cabinet performance. The degree of compromise for a given model can be estimated by measuring the effect of each selected condition on the air flow balance point and plotting the results on that model's performance envelope. This procedure can be used in the cabinet development process to select a nominal setpoint that will minimize the effects of the more common adverse laboratory conditions.

RECOMMENDATIONS

BSC performance envelopes have the potential to aid in three important areas: manufacturing, selection, and certification. The use of performance envelope data makes it possible for cabinet manufacturers to optimize BSC performance in existing and future models. It gives consumers another tool to use when selecting from among the various BSC models available for one to best fit their particular laboratory conditions. Users can have the certifier adjust the cabinet operational setpoint within the National Sanitation Foundation requirement of +5 fpm to favor either product or personnel protection, with confidence that the cabinet will continue to perform under the everyday laboratory conditions that can cause the air flow of the cabinet to vary.

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For further details on this topic, refer to the study published in Applied Occupational and Environmental Hygiene, Volume 5, Issue 6, page"s 370–377, June 1990.